[Proc. Roy. Soc. Victoria, **54** (N.S.), Pt. II., 1942.]

Art. XI.—*Further Observations on Rose Wilt Virus.*

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[Read 11th December, 1941; issued separately, 31st August, 1942.]

A disease of rose plants the primary symptom of which suggested the name "Rose Wilt" and the secondary symptoms the name "Rose Dieback" was shown in an earlier paper (Grieve, 1931) to be a sap transmissable virus disease. It was pointed out then that the disease assumed epidemic proportions only at considerable intervals and as a consequence no continuous study of it has been found possible. This paper, however, presents the results of some further observations and experiments. Since the writer's original paper appeared, three other virus diseases of rose plants have been described one of which has many features in common with Rose Wilt.

Review of External Symptoms of Rose Wilt.

Little modification of the original description of the disease is necessary, but it appears desirable to restate in summary form the symptom picture. The recurving or epinasty of the individual leaflets on an infected shoot so that they give the appearance of being closely balled together is a constant feature of considerable diagnostic value (Plate XIII., fig. 1). This abnormal condition of the infected leaves persists as long as they remain on the plant. With this reflexing of leaflets is associated a condition of increasing brittleness of the laminae. Abscission occurs very easily on touching or under the action of a gust of wind. On a badly infected plant complete defoliation may occur quite early, but generally a proportion of the recurved leaves remain attached for some time (Plate XIII., fig. 3). External lesions have not been observed in young leaves in the reflexed stage, but occasionally, when affected leaves remain on the plant, reddish-brown, bordered, necrotic spots appear on the leaves. The dying back of the young shoots subsequent to defoliation proceeds quite rapidly in many cases and within a few days the plant presents a scorched and blackened appearance. Where more mature shoots are affected, dying back proceeds more slowly and a mottled appearance is produced on the affected canes by the presence of brown to black areas interspersed with areas where the original green colour of the cane is retained. The discoloured areas become larger and coalesce until finally the whole shoot is quite

discoloured and black. It has frequently been observed that oval green patches remain surrounding the buds on the canes for some time after the rest of the cane is blackened.

In plants which in one season suffer only a mild attack, the leaves may show only slight recurving and defoliation does not occur. Flowers come to bloom on such plants and these flowers show no traces of deformation. Observation on such infected plants over two to three seasons has shown that the disease remains in them and may become serious in following seasons. On thick canes of these plants, reddish, somewhat raised areas are frequently scattered. It is of interest to note that the curious reflexing of the leaflets of virus-infected rose plants is paralleled by the effect on rose plants of ethylene gas (Zimmerman, Hitchcock, and Crocker, 1931), and to some degree also of a growth substance, β-indole-acetic acid (Grieve, unpublished data).

Experiments on the relation of auxin to epinastic response in several plants indicate that this growth movement is associated with a greater concentration of hormone toward the upper sides of the petioles. It appears probable in the case of Rose Wilt that one of the initial effects of the virus is to cause some similar disturbance in hormone concentration at the bases of the leaflets.

Internal Symptoms.—Histological Changes.

The internal symptoms associated with Rose Wilt have not previously been described, consequently the morbid anatomy of infected plants will be treated in some detail.

Examination of fresh stem sections of infected plants showed abnormalities of the cortical tissues and of the phloem similar to those which have been demonstrated in certain other viruses, such as Acropetal Necrosis of potato and Potato Leaf Roll.

Material for examination was fixed in Fleming's or Glacial acetic acid fixative, dehydrated and embedded in paraffin. Sections were cut at various thicknesses from 3 to 10μ. Haidenhain's iron alum haematoxylin with eosin counter stain, Fleming's triple stain and Safranin and Light Green were used for staining.

Healthy Stem.

For purposes of comparison a short account of a transverse section of a healthy rose shoot is here given. The stem is bounded by a single epidermal layer with a well-defined cuticle, beneath which there is a single sub-epidermal layer of fairly regular cells. The cortex consists of two types of cells, collenchymatous and parenchymatous. The collenchyma, which occurs beneath the sub-epidermal layer comprises a region varying from three to five cells in thickness. Many of these cells contain large deposits of tannin. The parenchyma zone is wider and consists of cells which vary from oval to rectangular in cross-section. The

innermost layer of the cortex bounding the stele was not found to differ in structure or size from the other cells of the inner cortex, but iodine staining showed it to be rich in starch and it may be considered as the endodermis. Within this endodermis is the pericycle which appears to be discontinuous: opposite each bundle it consists of several rows of thick-walled tightly-packed cells, while where the medullary rays pass out, the cells are parenchymatous. The vascular bundles are well defined and show considerable variation in size, both in a radial and in a tangential direction. The wood consists of vessels, tracheids and wood parenchyma; the phloem of sieve tubes and companion cells. The central part of the stem is composed of pith, two types of cell being present. Small thick-walled cells are found occurring in groups of two to five interspersed between much larger thin-walled cells. In the young shoots cambial activity is visible at an early stage and there is present secondary xylem and secondary phloem.

Rose Wilt Infected Stem.

Sections of shoots taken near the bases of reflexing leaves frequently showed as a first pathological condition the presence of considerable deposits of a brown gummy substance in and around the vessels. Slightly later, while the recurved leaves were still present on the cane, sections of petiole bases and of shoots showed necrosis of cells of the inner cortex, of medullary ray cells, and of a few cells of the secondary phloem. Affected cells in the cortical region were mainly opposite the bundles, but necrosed cells were also observed scattered through the cortex. Cells of the primary medullary rays were mainly affected in groups between the pericyclic fibres of contiguous bundles. The symptoms in these cells consisted of thickening and yellowing of the cell walls. Yellow gum-like deposits were present in necrosed cells.

At a slightly later stage when defoliation was commencing, sections of shoots showed greater necrosis of the phloem, the symptoms being thickening of the cell walls of the secondary phloem elements followed by the crinkling and collapse of cells (see Plate XIV., fig. 5, and text fig. 1). Owing to separation of cells from one another large intercellular spaces developed. Gum deposits were present in some of the affected cells and in the intercellular spaces.

Staining reactions indicated that the walls of affected cells were suberized while the deposits in the cells and intercellular spaces were gum-like. A red colour in the walls was obtained using Sudan III. and some of the deposits also showed a red colour. The necrotic regions resisted treatment by concentrated sulphuric acid. Tests with phloroglucin and hydrochloric acid gave negative results for lignin in necrosed cells; it was present in healthy cells of the collenchyma region of the cortex, the

thick-walled pericycle and the xylem. Sections of diseased tissue stained with iodine revealed a difference from healthy stems in that the endodermis contained little or no starch. Sections through shoots in which the dieback stage was commencing showed more severe necrosis in the epidermal and cortical cells as well as in the phloem. The necroses in the epidermal cells were associated with the discolouration visible externally at this stage. In some cases it was observed that necrotic cells could be traced from the epidermis to the pith via the primary medullary rays.

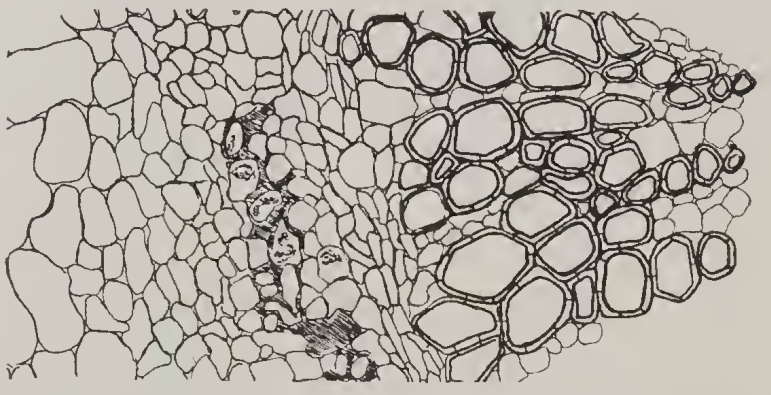

Fig. 1.—Transverse section through cane of infected rose showing necrosis of phloem. Some cells have collapsed, others have thickened walls and gum is present.

Rose Wilt Infected Leaf.

Young leaves in the reflexed condition showed no histological differences from healthy leaves. Examination of sections cut at thicknesses of 3–12μ and stained in a variety of ways failed to show the presence of intra-cellular inclusions. In the course of observations on the starch content of healthy and infected leaves, using the iodine staining technique, it was noticed, however, that in reflexed leaflets several minute colourless areas of pinprick size showed amid the general black stain due to the presence of starch. These small non-starch areas were not seen in healthy leaves. On close visual examination of fresh infected leaves, which were of comparable age to those iodine tested, no trace of any lesions could be detected. Examination of older leaves which had not dehisced showed that in some cases visible lesions were present. These were circular to irregular in shape, and of red-brown colour. Iodine staining showed an absence of starch around these spots. Sections cut through them revealed yellowing of palisade and of spongy mesophyll cells and the presence of gum-like deposits in affected cells (Plate XIV., figs. 2 and 3). Examination of cells in the vicinity of the necrosed regions showed the presence of a differential staining body generally situated close to the

nucleus (Plate XIV., figs. 6 and 7). These intra-cellular inclusions or X-bodies were oval or spherical in shape, and in some cases were comparable in size to the nucleus. The cells in which these were observed were mainly of the palisade layers fairly close to those showing necrosis or gum formation. They were present also in spongy mesophyll cells, but were not observed in epidermal cells. Owing to the presence of breakdown products in cells in a necrosed region, it was not possible to observe X-bodies in them. Nuclei in such cells were very much swollen as were also the chloroplasts.

Experiments on Transmission.

Mechanical Inoculation.

The disease is transmitted with difficulty, but it has been obtained in several cases by the following method. Infected leaves are ground in a little water to which fine emery powder is added and the extract pressed through cheesecloth. The virus containing juice is then rubbed over the surfaces of leaves of healthy plants, using the forefinger covered by a cheesecloth strip. Injection of virus juice using capillary tubes inserted into the canes near the bases and axils of petioles also produced infection. Using roses grown from cuttings, symptoms have been found to develop ten to twenty days after inoculation. Considerably greater difficulty was experienced in obtaining successful transmissions in rose plants growing in the open. In a group of 40 inoculations in the field only four proved successful. Controls remained healthy during the experiment.

Cross Inoculation Experiments.

Suitable members of the family Rosaceae were selected in attempts to transmit Rose Wilt by the mechanical inoculation method, but no clear-cut positive results were obtained. In the case of *Geum coccineum* and *Poterium sanguisorba* a suggestive recurving of the leaflets occurred in inoculated plants a few days after inoculation, but no further symptoms appeared.

To test a possibility that the symptoms of rose wilt might be really due to the virus of Tomato Spotted Wilt, a virus which produces diverse symptoms in a wide variety of horticultural plants, cross inoculations using expressed juice were made from infected roses to *Nicotiana tabacum* (White Burley Tobacco), *Nicotiana glutinosa*, and *Solanum lycopersicum* (Tomato variety Marglobe). No disease condition was produced by these inoculations. Conversely, it was not found possible to induce disease in roses by inoculation with Tomato Spotted Wilt virus. It is therefore concluded that these two diseases must be regarded as being caused by different viruses.

Transmission by Budding.

The procedure adopted was to take buds in mid-summer from canes of plants which were showing the dieback condition in a mild form in some shoots. Insertion of the buds into healthy plants was made towards the base of ripe shoots, using the T-bud method.

Considerable difficulty was experienced in getting the infected buds to take successfully owing, it is believed, to the progress of the disease in them. In the experiments so far concluded 40 healthy plants have been used. Of these twenty were budded, using buds from infected plants. The other twenty served as controls, being budded from healthy stock. In the test series union was successfully effected in only four plants, the infected buds in the other sixteen plants shrivelled and died. Of the four plants in which union was obtained two developed typical rose wilt symptoms. Buds inserted on the control plants took well and these plants remained healthy. Although the number of successful transfers was small, the fact that they were well controlled gives dependability to the result. Further experiments using the patch-grafting method are being tried to obviate the difficulty of working with buds weakened by the virus.

Experiments on Insect Transmission.

The spread of the disease in gardens during one of its periodic visitations strongly suggests that an insect vector is involved. In a search for such a possible insect transmitter a number of experiments have been carried out. Different species of aphides from diseased rose plants have been tested for infectivity, but the results so far have been to a large degree negative, and will not be reported in detail. It should be pointed out that the search has been of an exploratory rather than of an exhaustive nature and is being continued as opportunity permits.

Serology.

The technique of serology was applied to the study of Rose Wilt Virus to determine whether it was antigenic, that is, capable of stimulating the production of precipitating and other antibodies when injected into rabbits, and also to determine whether this virus and Tomato Spotted Wilt were serologically distinct. The wide host range in horticultural plants and the diverse symptoms produced by Spotted Wilt virus made this a desirable test. Antisera for Rose Wilt and Tomato Spotted Wilt were prepared, using essentially the methods of Chester (1935).

No virus specific precipitin reaction was obtained, however, in either case. Mushin (1942) working in this laboratory has, in the course of more extensive serological work, obtained the same results for Rose Wilt. Chester (1937) concluded that Tomato Spotted Wilt was non-reactive serologically. He pointed out that viruses which so far have been shown to be serologically inactive

have in common the characters of being difficult to transmit by mechanical means and of being relatively unstable in vitro. Though little is known as yet of the properties of Rose Wilt virus, the relative difficulty of its transmission by mechanical means and its serological inactivity makes it probable that it belongs to the group of viruses which are unstable in vitro.

Since neither Rose Wilt nor Tomato Spotted Wilt produce active sera they cannot be shown to be separate viruses by this means. As pointed out in an earlier section, however, Tomato Spotted Wilt virus could not be transmitted to Rose plants, and on this evidence, together with the fact that no X-bodies have been observed in Spotted Wilt infected plants, it is concluded that the two viruses are distinct.

Observations on Susceptibility of Rose Varieties.

In Victorian gardens the great majority of roses grown belong to the Hybrid Tea Group. Pernetianas, Hybrid Perpetuals, and Tea roses are also grown, but not in any abundance. Of these four groups observation has shown that the Pernetiana roses, or those roses with some of the Pernetiana strain in them, are most susceptible to Rose Wilt.

Golden Emblem and Ville de Paris are two outstanding examples of Pernet roses which take wilt badly. The popular Hybrid Tea roses, while as a class being somewhat more resistant than the Pernetianas, are nevertheless very subject to the disease. Some gradation in intensity of infection occurs. Rose Wilt Virus has been observed on the following Hybrid Tea roses in severe form:—Dame Edith Helen, Sunburst, Yvonne Vacherot, Mme. A. Chatenay, Columbia, Lorraine Lee, Mrs. McKee, and Etoile de Hollande. On the other hand, the varieties Sunny South and Chateau de Clos Vougeot appear to be more resistant to the disease.

Tea roses appear to have relatively the greatest resistance to Rose Wilt and, in fact, no certain cases of wilt on tea roses has been brought to the notice of the author.

Comparison of Rose Wilt with Other Rose Viruses.

Three virus diseases of rose other than Rose Wilt have been described, one in Italy and two in the United States of America. Rose Mosaic (White, 1932; Thomas and Massey, 1939; Brierley and Smith, 1940) and Streak of Roses (Brierley and Smith, 1940) in the United States of America appear to be quite distinct from Rose Wilt.

Gigante's (1936) new virus disease of roses in Italy has, however, many features in common with Rose Wilt, while there are also apparently certain minor points of difference. A comparison in summary form of external and internal symptoms of the two diseases is presented in Table 1, as are also symptoms of Streak and Rose Mosaic. Symptoms which Rose Wilt and the

Italian Rose disease have in common are the very characteristic recurving and balling of young leaflets, followed by defoliation from the apex downwards. This again is followed in both diseases by the browning and blackening of defoliated canes. Primary symptoms, such as the presence on the leaves of dark spots, and yellowish-brown necrotic areas surrounded by a blackish halo, as described by Gigante, do not occur in the Australian Rose disease. The presence of leaf spotting may occur some considerable time after "balling" and when defoliation fails to occur. Gigante also stresses the early appearance on infected canes of reddish-brown raised areas. As indicated earlier, such raised spottings have sometimes been observed on canes of plants which have been infected with the Rose Wilt for more than one season, and in which a certain degree of recovery from the disease is apparent.

TABLE 1.—COMPARISON OF SYMPTOMS OF ROSE VIRUSES.

——	Rose Wilt Virus.	Gigante's New Italian Rose Disease.	Streak Disease.	Rose Mosaic.
External Symptoms	Recurving and balling together of leaflets on the petioles. Leaves become brittle and defoliation occurs commencing at the apex and working downwards. After defoliation rapid dying back of the canes occurs	Leaflets recurving and balling. Blistering of leaves and the development of small dark spots on the leaves. Reddish lesions on the canes. Defoliation working from apex to base. Flowers show deformation	Brownish rings and brown vein banding. Water soaked ring patterns in canes	Rose Mosaic 1. (Thomas and Massey) Small chlorotic spots somewhat angular or fringed in appearance. Distortion of leaf blade. Flowers deformed and pale in colour. Rose Mosaic 2. Chlorotic lines, bands and broad blotches in the leaf blade with or without distortion Rose Mosaic 3. Broad chlorotic blotches in the leaf blade. Dwarfing of the plant
Internal symptoms	Necrosis of cortex, medullary ray cells and of the phloem. Suberization of walls and gum formation. Intracellular inclusions present in leaves	Necrotic cells present in stem parenchyma. Necrosis of cells in the leaf. Intracellular inclusions present	None recorded	None recorded
Transmission	Mechanical inoculation and budding	Mechanical inoculation. Insect vector believed to be a species of *Macrosiphum*	Budding and grafting	Budding and grafting

In so far as the histology of the two diseases is concerned there is agreement with Gigante as to the necrosis of cells of the cortex and the medullary rays of infected canes. The formation of cork cells in the cortex cutting off the necrotic cells, which he records, has not, however, been seen in the case of Rose Wilt. Gigante makes no reference to necroses of the phloem in the Italian rose disease, and it is not possible from the photomicrographs in his paper to tell whether any is present.

Intra-cellular inclusions have been found in diseased leaves in both viruses. Gigante finds them, as does the author, in leaves which are showing some necrosis of cells. In the case of the Italian rose disease it appears that the leaf lesion symptom and X-bodies occur early, whereas in Rose Wilt, necrosis of cells has been found only in those leaves which remain attached for a long time, and X-bodies cannot be seen in the balled-up leaves which abscise early.

Both viruses are sap transmissible with difficulty, and there is evidence that Rose Wilt is transmitted by budding. The insect vector in the Italian rose disease is believed to be a species of *Macrosiphum*, but no conclusion has been reached regarding an insect vector in the case of Rose Wilt.

It would appear, despite minor symptom differences, that the viruses are the same. Confirmation of this must await further information on the properties of the viruses and the discovery of useful differential hosts.

Control.

The view was earlier expressed (Grieve, 1933) that infection might be carried by secateurs during pruning operations on healthy and virus-infected plants. The relative difficulty of transmission by mechanical inoculation leads one now to consider that such a method of spread is unlikely in practice. Nevertheless, sterilization of secateurs in dilute formalin remains a useful general precaution.

The experiments on budding have shown that in practice this is a possible method of transmission of Rose Wilt. Badly infected plants as a source of buds are of course not in question. The danger lies in utilizing rose plants which have been only slightly affected or have made an apparent recovery.

Evidence as to the relative susceptibility of rose types to wilt is incomplete. Members of the Pernetiana, H.T. and H.P. groups have all been found subject to the disease. Those varieties in which the Pernetiana strain has been incorporated do show a greater degree of susceptibility, and should be avoided.

The rapid and random spread of the disease in gardens during certain seasons suggests that an insect vector is concerned, although none as yet has been implicated. The only effective remedy against spread at the present time is the removal and burning of the infected plant as soon as the symptoms become manifest.

Summary.

1. The external symptoms of Rose Wilt virus are briefly reviewed and an account is given of the morbid anatomy of infected plants. Necrosis of cells occurs in the cortex, medullary rays, and in the phloem of the canes, as the disease progresses

from the stage of reflexing of leaflets to their defoliation. Microchemical tests indicated that the necrosis was accompanied by suberization of walls and the secretion of a gum-like substance.

2. Intra-cellular bodies were found associated with the nucleus in leaves which remained for some time on the plant. In the majority of cases the leaves absciss shortly after becoming reflexed and before lesions develop. At this stage no X-bodies were seen.

3. Results of experiments on transmission by mechanical methods and serological evidence suggest that Rose Wilt is an unstable virus. In a small percentage of cases successful transmission by T-budding was effected.

4. Control measures are discussed in the light of the experimental results obtained. The author is indebted to Prof. Woodruff for help and for facilities for the serological tests.

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Explanation of Plates.

Plate XIII.

Figs 1 and 2.—Virused plants with reflexed and balled-up leaves.

Fig. 3.—Artificially infected plant showing both reflexing and defoliation stages.

Fig. 4.—Infected plant approaching the die-back stage of disease.

Plate XIV.

Fig. 1.—Transverse section through leaf. X—bodies in association with nuclei. X = intracellular body, n = nucleus.

Fig. 2.—Section of leaf showing the origin and spread of the necroses.

Fig. 3.—Section of leaf magnified to show necrotic palisade cells on left.

Fig. 4.—X—body in association with the nucleus in a cell of the spongy parenchyma.

Fig. 5.—Section of the cane showing advanced necrosis of the Phloem. Some of the cells are obliterated and others are filled with gum.

Fig. 6.—Palisade cells of leaf showing presence of X—body.

Figs. 7 and 8.—High power magnifications from section in Fig. 1 to show intracellular inclusions.

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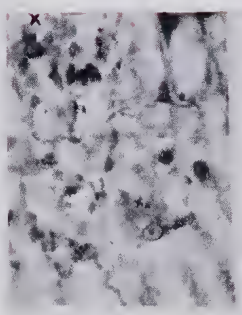

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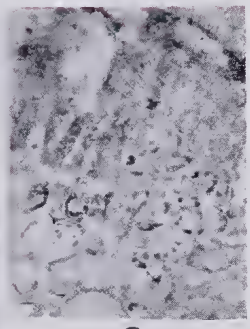

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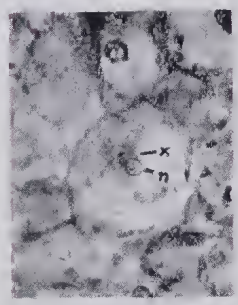

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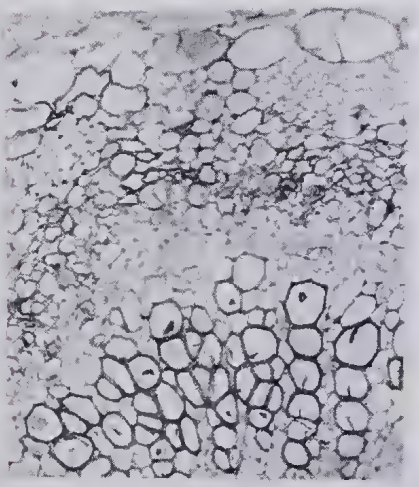

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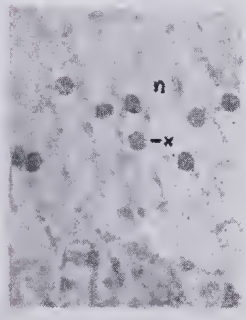

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